REMARKS

Rejections under 35 U.S.C. §103

The Office Action rejects claims 20-24 under 35 U.S.C. §103(a) as obvious over Balaraman *et al.* (US 5,434,059) in view of Mizutani (US 4,009,264) and Hanson *et al.* (5,700,669). Applicants respectfully traverse.

The present invention is directed to isolating a novel enzyme, thrombinase, with unexpected properties of having a 31,000-32,000 Daltons molecular weight range. A thrombinase having such properties has not previously been reported or isolated and was not envisioned, disclosed, or suggested by Balaraman *et al.* (which isolated an enzyme of 18,500 Daltons). Although Balaraman *et al.* isolated a member of the thrombinase enzyme family, the enzyme has different properties such as lesser efficacy, has a molecular weight that is almost half, was less pure, and produced a lower yield than the one isolated in the present invention, which obtained an enzyme family member previously unknown to those skilled in the art (see paragraphs 0001 and 0002 of the present invention).

Applicants maintain that the Office Action fails to establish *prima facie* obviousness. The references, even when combined, fail to teach each and every element of the claims. In particular, the prior art fails to teach, disclose, or suggest thrombinase having a molecular weight of 31,000-32,000 Daltons, much less a method of isolating such a thrombinase. The prior art also fails to teach the combination of steps and conditions as claimed and there is no motivation, either in the references or in the general knowledge of the art, to modify the references in a way that teaches the claimed method. Finally, there is no expectation of obtaining a thrombinase of the molecular weight as claimed.

Although the process steps detailed in claim 20 of the present application describe a fermentation process that is known *if the steps are considered individually*, the combination of the specified ratio of each of the ingredients in the fermentation medium

used, the specific pH range, the use of two-step ultra-filtration using two specifically sized membranes (first membrane of 100,000 Daltons then a second membrane of 10,000 Daltons) to isolate the enzyme family member claimed, the specific concentration of ammonium sulphate, and the use of ice-cold acetone for re-precipitation yielded a *synergistic effect, which resulted in the unexpected production of the claimed novel thrombinase* of 31,000-32,000 Daltons in size.

In adherence to USPTO rules, when interpreting a claim, the entire matter contained in the claim has to be taken in totality and not considered part by part. Thus, if one interprets the combination of steps of claim 20 of the present invention, the specific steps, reagents, and conditions, for obtaining the resulting novel thrombinase would not be obvious to a person of ordinary skill in the art. Further, applying the methods disclosed by Balaraman *et al.*, even if modified as suggested by the secondary references, would not result in the successful isolation of the presently claimed novel thrombinase of 31,000-32,000 Daltons. Thus, the differences in methods between the present invention and those of Balaraman *et al.* yield two different enzymes and would not allow for the isolation of the presently claimed thrombinase. The concluding statement that "to obtain a thrombinase between the range of 31,000-32,000 Daltons is also well within the purview of an ordinary artisan" is unsubstantiated and insufficient. Without some evidence that such an entity exists, obtaining it cannot be within the purview of the art.

The Office Action has not adequately or correctly considered the state of the art that describes the methods of isolating thrombinase. The state of the art teaches that one would *not* expect to isolate an enzyme of this size (31,000-32,000 Daltons) using the conditions and reagents disclosed by the Balaraman *et al.* method. The prior art does not discuss methods for isolating the claimed novel thrombinase of 31,000-32,000 Daltons, which is an enzyme that was previously unknown in the art. In fact, the claims and details of the method disclosed in Balaraman *et al.* are specifically limited to isolating a known and much smaller enzyme of 18,500 Daltons.

Additionally, the change in size of the membrane filters of the present invention (namely, filter of 100,000 Daltons then of 30,000 Daltons) and those used by Balaraman *et al.* (namely, filter of 30,000 Daltons then of 10,000 Daltons) are not trivial but

substantial. One skilled in the art would not be motivated to experiment with membrane filters of different sizes in unsubstantiated attempts to obtain a novel enzyme. The present invention, on the other hand, utilized a novel combination of method steps to obtain an unexpected result (namely, isolation of a novel thrombinase of 31,000-32,000 Daltons). For these reasons as well, *prima facie* obviousness is not established and the rejection should be withdrawn.

Further, as explained below, the combination asserted by the Office Action does not render the claims obvious because a person skilled in the art would not combine the references as set forth and, even if combined, there would be no reasonable expectation of success. Accordingly, the rejections should be withdrawn.

The Office Action states that Mizutani (which discloses making complexes of polysaccharides or their derivatives with reduced glutathione) teaches product collection by precipitation with acetone and by a repeated cycle of dissolving the precipitate in water and reprecipitation in acetone, ending in purification of the final precipitate. However, a person of ordinary skill in the art would recognize that different additives are utilized in enzyme isolation as compared to the products isolated in Mizutani. It is not the use of a known precipitation media but its use in combination with the other presently claimed steps, which are not disclosed by Mizutani, that distinguishes the present invention. Just because steps can be combined, does not mean that one skilled in the art would have combined them at the time that the invention was made. ("The mere fact that references *can* be combined or modified does *not* render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art"; MPEP 2143.01 section III, emphasis added.)

Further, utilizing *ice-cold* acetone is described neither by Mizutani nor by others, including Balaraman *et al.*—and this modification yields a unique result when taken in combination with the other steps disclosed in the present application. Specifically, using ice-cold acetone facilitates retention of thrombolytic activity and recovery of the novel thrombinase during re-precipitation. A person seeking to isolate a novel thrombinase of 31,000-32,000 Daltons would, therefore, not look to Mizutani in seeking to modify or improve the Balaraman *et al.* method.

The Office Action states that one skilled in the art would utilize the culture media of Hanson *et al.* and choose to cultivate *Bacillus sphaericus*. First, Hanson *et al.* is directed to the preparation of taxanes, which are very different from the enzymes isolated by the present invention. However, such uses have been made as a general description by others, none of whom describe the methods and isolation of a novel thrombinase of 31,000-32,000 Daltons as disclosed in the present invention. A disclosure that some culture media contain additional components does not, by itself, make the reference combinable with Balaraman *et al.* in a manner that renders the claims obvious. A proper analysis of Hanson *et al.* reveals that one skilled in the art would not be motivated to combine the methods Hanson *et al.* uses to prepare taxanes with Balaraman *et al.*, which isolates an enzyme.

As previously described, the enzyme of Balaraman *et al.* is not only a significantly smaller thrombinase than that claimed by the present invention but also fails to achieve the higher yield and higher purity of the presently claimed methods. Since the patents of Mizutani and Hanson *et al.* are directed to very different types of reactions and isolation steps, one skilled in the art would not look to these references to modify methods of Balaraman *et al.* Modifying the method of Balaraman *et al.* in view of Mizutani and Hanson *et al.*, as asserted by the Office Action, would not be expected to change the results. Thus, as presented in the afore-mentioned arguments, if one combined the teaching of Mizutani and Hanson *et al.* with Balaraman *et al.*, the modification would be insufficient for the isolation a novel thrombinase of 31,000-32,000 Daltons and, in fact, would not work for its intended purpose. Accordingly, the rejections should be withdrawn because a person skilled in the art would not combine the references as set forth and, even if combined, there would be no reasonable expectation of success.

None of the references, whether taken alone or combined, disclose or teach the claimed method for isolating a novel thrombinase of 31,000-32,000 Daltons. Thus, the Office Action fails to establish *prima facie* obviousness because the references do not teach each and every limitation of the claims. The examiner's assertions that the methods disclosed in Balaraman *et al.* combined with the culture media of Hanson *et al.* and the precipitation with acetone of Mizutani do not remedy this situation. As detailed in the

arguments presented by the Applicants, one would not expect to obtain a novel thrombinase of 31,000-32,000 Daltons by applying Balaraman *et al.* method since unique additional steps and specific conditions (e.g., different culture media, re-precipitating in ice-cold acetone, using a specific pH and range of ammonium sulphate, and using specifically sized membrane filters) are used in the presently claimed method.

CONCLUSIONS

Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. Accordingly, Applicants request that the Examiner issue a Notice of Allowance for pending claims 20-24 and that the application be passed to issue. Applicants respectfully request that a Notice of Allowance of pending claims 20-24 be timely issued in this case.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is hereby invited to telephone the undersigned at the number provided. The Commissioner is authorized to charge any deficiency in any patent application processing fees pursuant to 37 CFR §1.17, including extension of time fees pursuant to 37 CFR §1.17(a)-(d), associated with this communication and to credit any excess payment to Deposit Account No. 22-0261.

Respectfully submitted,

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